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2022.07.19 Project Skunkworks: primerless extension PCR for RadegenBio *de novo* DNA oligo synthesis process

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Creative Business Concept: Radegen Biotechnology's DNA synthesis process relies on the building of a DNA oligonucleotide molecule of a specified sequence by a *de novo* enzymatic stepwise process (*Dtd+* [1]). The resulting fragment is a 5' tethered ssDNA fragment. The sequence identity of this fragment is designed to be the reverse complement of the desired sequence.

The next step in the process consists of primerless extension PCR with the use of a primer independent polymerase (*NrS-1* DNA Pol) (2). Next the desired fragment developed from the reverse complement bead tethered template is collected by NaOH denaturing. The collected material can be used as a primer or made into a duplexed DNA fragment using (*NrS-1* DNA Pol).

This polymerase is a key component to Radegen Biotechnology DNA synthesis (DNA forging) platform. It provides the DNA synthesis systems the ability to be independent of chemically synthesized oligos. An initial ssDNA preparation can be simply PCR amplified from a random vector sequence using premiers with a 5' biotin modification (a common tool employed in Molecular Microbiology) for tethering to a substrate.

1. https://archive.org/details/2022.03.11-skunkworks-mol.toolkit.cc-by-nc-sa-4.0
2. 2017. Proc Natl Acad Sci USA. Mar 21;114(12)E2310-E2318. PMID: **28265063**